Amendments to the drawings

The attached sheet(s) of drawings have been amended to include numbered reference to parts.

Attachment: replacement sheets

REMARKS

Claims 55 – 65, 67, 71, 77 – 79, 81 – 83, 85, 87 – 91 and 103 – 105 are pending in the application. Claims 1 – 54, 63, 66, 68 – 70, 72 – 78, 80, 82, 84, 86, 88, 92 – 102 have been cancelled. Claim 90 has been amended. New claim 106 has been added. No new matter has been added by virtue of the amendments and claims, support being found throughout the specification and claims as originally filed.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

Drawings

Applicants have amended the drawings in compliance with 37 CFR 1.121. An Appendix including amended drawing figures is attached.

Claim Rejections

35 USC 112, Second Paragraph

The Examiner has rejected claims 55 – 62, 64, 65, 67, 71, 79, 81 – 83, 85, 87 – 91 and 103 – 105 under 35 USC 112, second paragraph for alleged indefiniteness. Applicants respectfully traverse the rejection.

The Examiner argues that "claims 55 and 60 are still confusing and ambiguous in their recitation of the claimed microfluidics system, and the structure of the claimed apparatus still cannot be understood, rendering the meaning of the claims unclear." (Office Action, p.2). The Examiner is still unclear as to whether the claimed device is a one-piece structure or a two-piece structure. The Examiner is unclear "(i)f the device is a two-piece structure, is the substrate the base…or is it the lid for the base or is it both?" (Office Action, p.2). Applicants respectfully disagree.

The device, as claimed, is a one piece structure. This is clear from the Figures and description in the specification. However, in order to further clarify the claimed microfluidic system, Applicants provide figures with numbered parts for reference.

The present claims recite a microfluidic system comprising a substrate, wherein the substrate comprises at least one measurement chamber for containing one or more cells or lipid based cell structures, the measurement chamber comprising at least one raised aperture for detecting an electrical property of one or more cells or lipid based cell structures, each aperture comprising a tip, the tip comprising a housing defining a lumen, wherein at least one tip is inserted into a cell or lipid based cell structure, and wherein the substrate further comprises at least one microchannel with at least one inlet and at least one an outlet which opens into the at least one measurement chamber, and wherein the at least one microchannel delivers one or more substantially separate aqueous streams into the measurement chamber.

Accordingly, the structure of the claimed microfluidic system is clear as claimed. Applicants direct the Examiner to Figure 10B, below, which shows an enlarged view of the measurement chamber 7 and the insertion of nanoelectrode 1 into the cell as it is exposed to solution flow from microchannel 8 to clearly illustrate an embodiment of the invention as claimed. Figure 10 B illustrates substrate 5 in the side view, clearly showing the relationship between the substrate, the measurement chamber, the microchannel, the cell and the nanoelectrode.

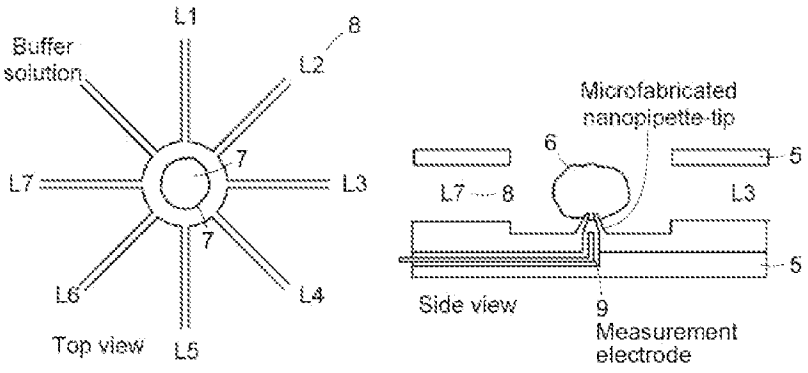

FIG. 10B

Accordingly, the structure of the claimed device as a one piece device is clear from the description and figures as filed.

The claims clearly recite a device where the elements, i.e., the substrate, the microchannels, the measurement chamber, the aperture are related, such that "the **substrate comprises at least one measurement chamber**…**the measurement chamber comprising at least one raised aperture**" and where "the substrate further comprises **at least one microchannel with at least one inlet and at least one an outlet which opens into the at least one measurement chamber**." (see claim 1). The specification provides description of the claimed device:

> the invention provides a microfluidic system comprising **a substrate that comprises a measurement chamber for containing one or more cells**. A plurality of **microchannels are fabricated on the substrate whose outlets intersect with, or feed into, the measurement chambe**r. Preferably, the system comprises one or more of the nanoelectrode devices described above. (p.47, emphasis added).

Claim 60 recites a microfluidic system comprising a substrate, wherein the substrate comprises at least one measurement chamber for containing one or more cells or lipid based cell structures, the measurement chamber comprising a plurality of solid electrode tips for detecting an electrical property of one or more cells or lipid based cell structures, the tips comprising a housing defining a lumen, where the housing comprises a solid state conducting material, wherein at least one tip is inserted into a cell or lipid based cell structure, and wherein the substrate further comprises at least one microchannel with at least one inlet and at least one an outlet which opens into the at least one measurement chamber, and wherein the at least one microchannel delivers one or more substantially separate aqueous streams into the measurement chamber.

Applicants direct the Examiner to the specification, beginning at page 43, where this aspect of the invention is clearly taught:

> Therefore in one aspect, the invention provides a system comprising **a substrate that comprises a plurality of measurement chambers** (e.g. greater than 10 and preferably 96, 384 or 1536 number of chambers). Each chamber on the substrate is **containing one or more cells**…In addition each measurement chamber is equipped with **at least one nanoelectrode device fabricated on the base of the measurement chamber** as described above. Most preferably, **each measurement chamber is equipped with multiple (e.g., greater than 6 and preferably greater than 30) nanoelectrodes** allowing the registration of several cells in each measurement chamber simultaneously. (emphasis added).

Accordingly, Applicants submit that claim 60 is also clear as written.

The Examiner argues that "amended claim 90 is somewhat clearer than its previous version, as the limitation of 'translating cells across the microchannels' has been deleted. But the limitation of 'scanning a cell across the aqueous streams from the microchannels' is still unclear and confusing." (Office Action, p.3). Applicants disagree.

Present claim 90 depends from the system according to claim 55 or 60, and further comprises a scanning mechanism, wherein the cell or lipid based structure is mechanically scanned across stationary microchannel outlets, wherein the microchannel outlets are mechanically scanned relative to a stationary cell or lipid based structure, or wherein a fluid stream is scanned across an immobilized cell or lipid based structure.

This is shown in the Figures, for example in Figure 18, described on page 28 – 29 where:

> Figures 18A-I are top views of microchannel outlets in relationship to a nanoelectrode 1-contacted cell, 6 collectively showing different methods by which a nanoelectrode-contacted cell can be moved in relation to the fluid streams. Figures 18A-C show mechanical scanning of the nanoelectrode-contacted cell across

stationary microchannel outlets. Figures. 18D-F show mechanical scanning of microchannel outlets relative to a stationary nanoelectrode-contacted cell. Figures 18G-I show a method for sweeping fluid streams across an immobilized nanoelectrode-contacted cell by controlled variation of the pressure across, and flow rates through, each individual microchannel.

The Examiner argues further that "(t)he claim may mean that the microfluidics system further comprises a scanning apparatus that scans cells or scans for cells by scanning across the width of one or more outlet channels (and) it is not clear, however, if the scanning is meant to create an image of the cell or to detect whether or not a cell is present at a particular point in time." (Office Action, p.4).

In addition to the description above at pages 28 – 29, Applicants point out that the specification clearly teaches that the scanning mechanism of the present invention is used for movement of the position of a cell of lipid based structure; nowhere does the specification teach or suggest that the scanning mechanism is an imaging or detection device. For example, at page 13, line 27 the specification teaches that the scanning mechanism is for "changing the position of a cell relative to the outlets of the microchannels. The scanning mechanism can translate the substrate containing the microchannel outlets relative to a stationary cell, or can translate the cell relative to a stationary substrate, or can move both cell and substrate at varying rates and directions relative to each other." For example, Figure 17 shows "chip designs for carrying out cell scanning across ligand streams using buffer superfusion to provide a periodically resensitized sensor, where FIGS. 17B-G show enlarged views of the outlets of microchannels and their positions with respect to a superfusion capillary and a nanoelectrode-contacted cell, as well as a procedure for carrying out cell superfusion while scanning a nanoelectrode-contacted cell across different fluid streams." (page 28).

In light of the above, Applicants respectfully request that the Examiner withdraw the rejection.

Rejection of Claims Under 35 USC 103(a)

The Examiner has maintained the rejection to claims 55 – 62, 64, 65, 67, 71, 79, 81 – 83, 85, 87 – 91 and 103 - 105 under 35 U.S.C. § 103(a) as being unpatentable over Maher et al. (US 2002/0025568; the '568 reference herein) and He et al. (US 2003/0049862; the '862 reference herein) in view of Peeters (US 6,123,819; the '819

reference herein) and Hamill et al. (Pflugers Archiv 391: 85 – 100, 1981). Applicants respectfully traverse the rejection.

The claims were set forth above.

The '568 reference is directed to instruments and methods for manipulating membrane potentials of living cells via electrical stimulation. The '568 reference teaches a method of assaying ion channel activity comprising exposing at least one cell to a plurality of electric field pulses so as to create a controlled change in transmembrane potential and activate an ion channel of interest, and detecting ion channel activity by detecting one or more changes in transmembrane potential without using a patch clamp.

The Examiner argues that the apparatus taught by the '568 reference "comprises a substrate comprising an array of measurement chambers (a microtiter plate) that contains cells. The apparatus comprises an array of microelectrodes that match the wells in the microtiter plate and are arranged in a lid or cover." (Office Action, p.5). As pointed out by the Examiner, the '568 reference uses "two identical, flat electrodes with surfaces that are aligned substantially parallel to each other" within the wells of the microtiter plates (paragraph [0147]). This is shown, for example, in Figure 9, below:

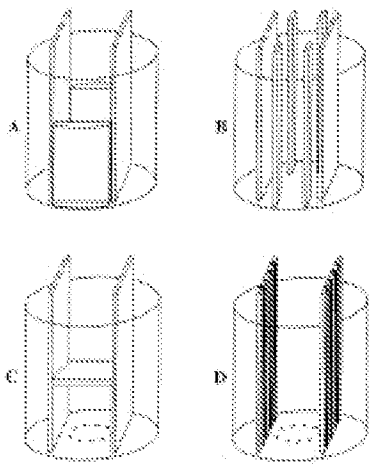

FIG. 9

Figure 1 (A and B), below, exemplifies the apparatus of the '568 invention, and shows the placement of the electrodes in the array. (see, e.g. paragraph [0155]). According to preferred embodiments of the '568 reference the "electrodes typically

consist of one or more pairs of electrodes that are arranged in an array that can be retractably moved into, and out of, one or more wells of a multiwell plate." ([0164])

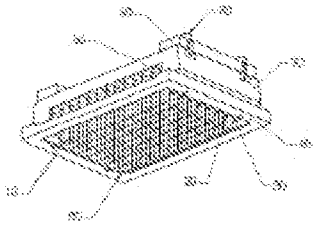

FIG. 1A

FIG. 1B

The present invention is directed to microfluidic substrates that comprise at least one microchannel with at least one inlet and at least one outlet which opens into the at least one measurement chamber. The microfluidic substrate comprises one or more microchannels which deliver substantially separate aqueous streams into the measurement chamber. Applicants direct the Examiner to the present disclosure, for example at page 56, beginning at line 3, that teaches the delivery of substantially separate streams to the measurement chambers.

> The system also can sweep different fluid streams across stationary cells by varying pressure drops across individual microchannels of the substrate. This design is derived from the discovery of a new and unique fluidic behavior; i.e., **that lateral interactions and couplings between neighbouring fluid streams as they exit from a set of closely spaced microchannels into a measurement chamber can extend dramatically the distance over which these streams remain collimated**. The second design exploits the reversibility of fluid behavior at low Reynold's numbers while the third design is based on the ability to rapidly exchange fluids in microchannels and chambers.

The '568 reference does not teach or suggest a substrate that comprises at least one measurement chamber with at least one raised aperture, where the substrate further comprises at least one microchannel with at least one inlet and at least one an outlet which opens into the at least one measurement chamber, and wherein the at least one microchannel delivers one or more substantially separate aqueous streams into the measurement chamber.

The '862 reference is directed to a microfluidic device that integrates fluidic capability into existing multi-well plates. The '862 reference teaches a device that includes "fluidic modules extending at an angle, preferably orthogonal, from a support structure or plate (and) (e)ach fluidic module is three-dimensional, has a major surface located remotely from the support structure, and at least one sidewall between the major surface and the support structure." ([0007]) There are fluidic channels in each of the fluidic modules.

The Examiner argues that "it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the microtiter plate of Maher et al. with the microfluidics plumbing for microtiter plate lids of He et al., because He discloses that this modification transforms the apparatus into high-throughput apparatus… (m)icrofluidics chips, by comparison, require specialized custom equipment and have much lower throughput." (Office Action, p.5).

The '862 reference does not cure the defects of the '568 reference.

The Examiner argues that "the rejection is one of obviousness over the combination of the cited references…(and) that it would have been obvious to modify the apparatus of (the '568 reference) with the microfluidics plumbing of (the '862 reference) to arrive at the claimed invention." (Office Action, p.6).

It would not be possible to use the plumbing taught by the '862 reference, where "the arrangement of the fluidic channels in each fluidic module permits one to supply a continuous flow of fresh reagents and solutions into each microplate well (and) …continuous fluidic movement is useful for such functions as mixing, flow-through washes or filtration" in the system of the '568 reference and arrive at the present

invention, where the substrate comprises at least one microchannel with at least one inlet and at least one an outlet which opens into the at least one measurement chamber, and the at least one microchannel delivers one or more substantially separate aqueous streams into the measurement chamber.

Neither the '819 reference nor the Hammil reference cures the defects of the '568 or '862 references. Neither reference, alone or in combination with any of the other cited references, teaches a microfluidic system as claimed.

In particular, the '819 reference does not teach or suggest a microfluidic system as claimed with raised apertures or a plurality of solid electrode tips (e.g., nanotips), where the tips comprise a housing defining a lumen and/or are inserted into a cell or lipid based cell structure. The '811 reference is directed to nanoelectrode arrays that are used for detecting small biological molecules. The '819 reference nowhere teaches tips that comprise a housing defining a lumen and wherein at least one tip is inserted into a cell or lipid based cell structure.

Applicants direct the Examiner to the description of the nanoelectrodes, at column 5, beginning at line 20, where the "(e)lectrodes 26a, 26b and 26c can be formed of a number of materials, either intrinsic or doped, such as gold and platinum and copper and other electrometals." Further, the '819 reference teaches only binding to the electrodes, where "several types of binding or adsorption of the molecule to the nanoelectrode receptor are possible, depending on the chemical composition of the nanoelectrodes, the voltage and the chemical to be measured. Binding forces may include covalent binding, electrostatic binding, hydrogen bonds and van der Waals bonds." (col 6, line 13). Nowhere does the '819 reference suggest any insertion of electrodes into cells, nor would the composition or arrangement of the electrodes facilitate such use.

In view of the above, Applicants respectfully request the withdrawal of the rejection.

CONCLUSION

In light of the above remarks, Applicants respectfully request early consideration and allowance of the subject application.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned attorney would appreciate the opportunity to do so.

The Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment to Deposit Account No. **04-1105**.

Respectfully submitted,

Electronic Signature:/Jonathan M. Sparks/
Jonathan M. Sparks, Ph.D.
Registration 53,624
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5543
Attorneys/Agents For Applicant